21ST

PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024
Kuala Lumpur
Malaysia

PGT and BEYOND...

Karyomapping and its Discontents

How Karyomapping Will Survive (And Thrive) In the Genomic Era

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Disclosures

Employee: Monash IVF Group
Advisor: Ex Ovo Genomics
Speaker: Ferring Pharmaceuticals, Vitrolife, Illumina, Organon

"It sounds like a fairy tale…this story of what man by his science and practical inventions has achieved on this earth."

Sigmund Freud, 1930

The Invention of Karyomapping

PGT and BEYOND...

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The Invention of Karyomapping

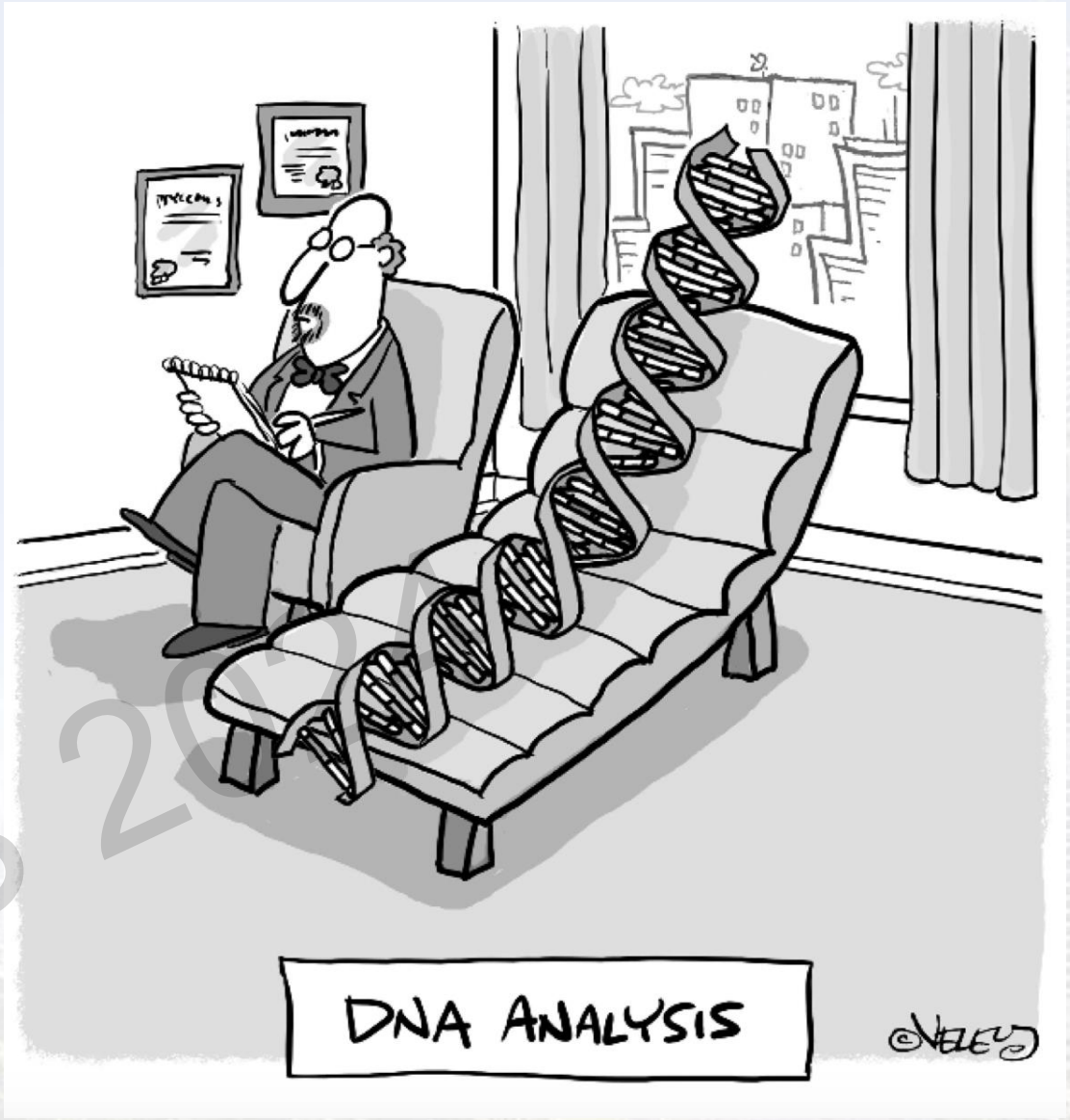
Genome-wide haplotyping using SNP array was a key advance in PGT:

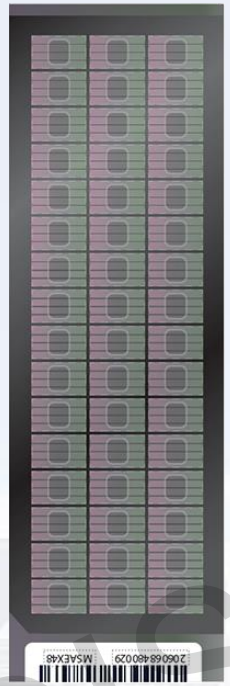
- Reduced the complexity of test design for monogenic conditions
- Championed PGT-A as a standard component of PGT-M
- Provided robust quality in the laboratory for a wide range of molecular pathologies

"Man has become a god by means of artificial limbs…but they do not grow on him and they still give him trouble at times."

Sigmund Freud, 1930

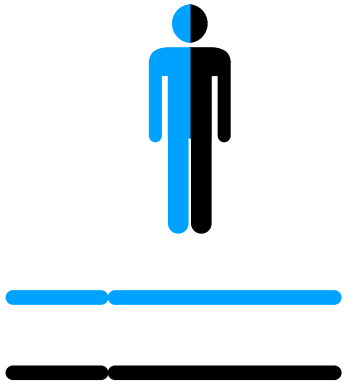
In the genomic era, does karyomapping face a crisis of confidence?



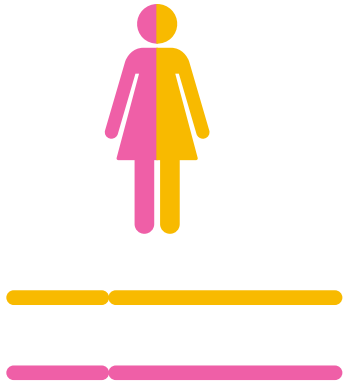


What happens if you perform karyomapping on a NovaSeq?

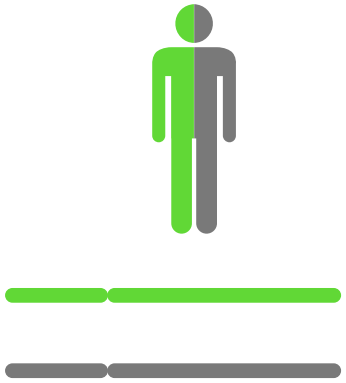
Paternal Grandfather
NA12889



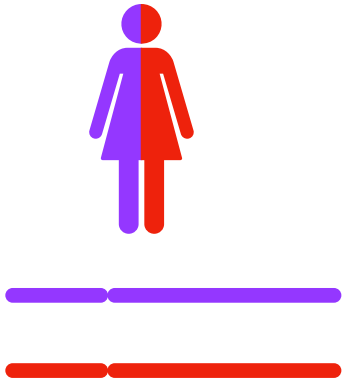
Paternal Grandmother
NA12889



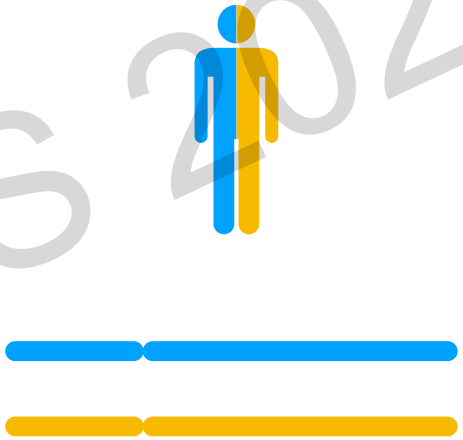
Maternal Grandfather
NA12891



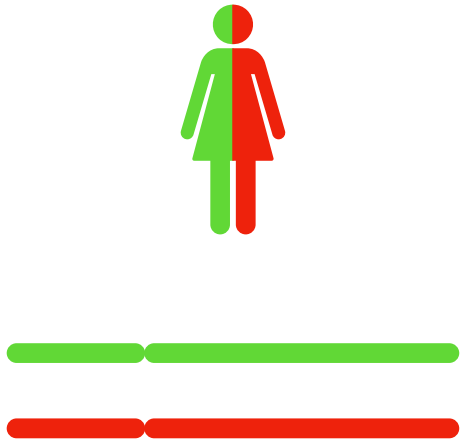
Maternal Grandmother
NA12892



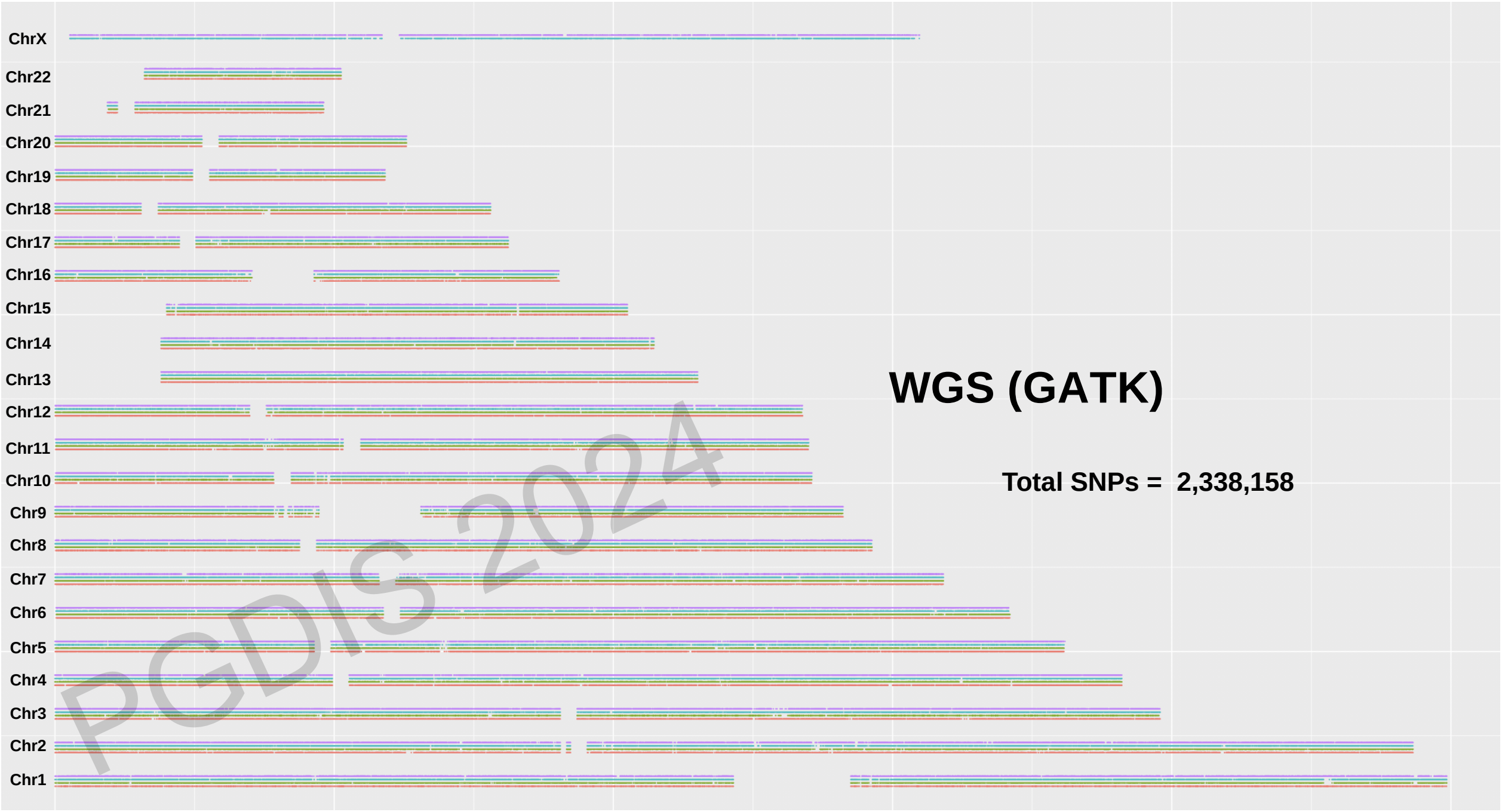
Father
NA12877



Mother
NA12878




ChrX
Chr22
Chr21
Chr20
Chr19
Chr18
Chr17
Chr16
Chr15
Chr14
Chr13
Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
Karyomapping Array
Total SNPs = 85,053
PGDIS 2024


ChrX
Chr22
Chr21
Chr20
Chr19
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Chr14
Chr13
Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
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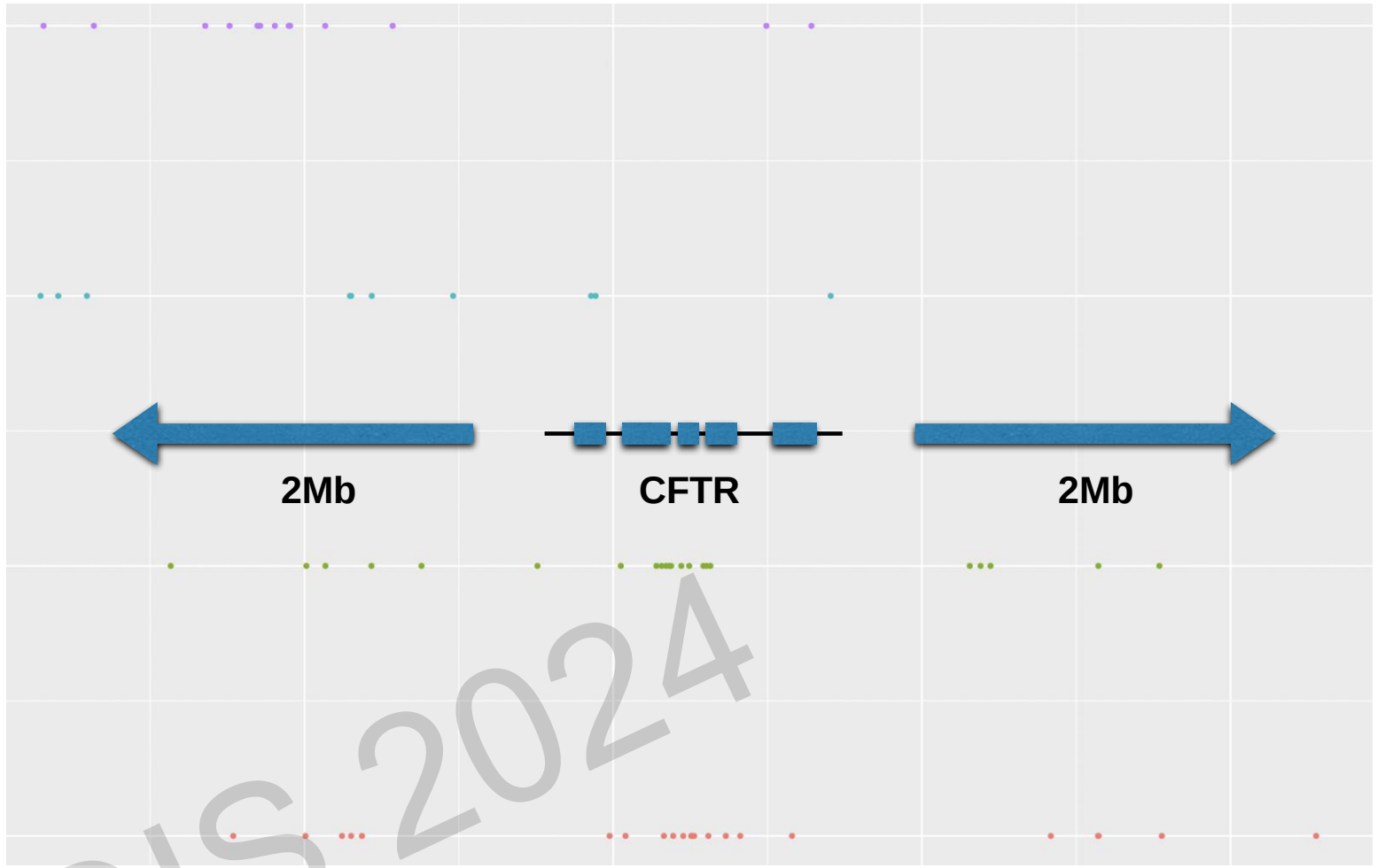
Chr5
Chr4
Chr3
Chr2
Chr1
WGS (GATK)
Total SNPs = 2,338,158
PGDIS 2024

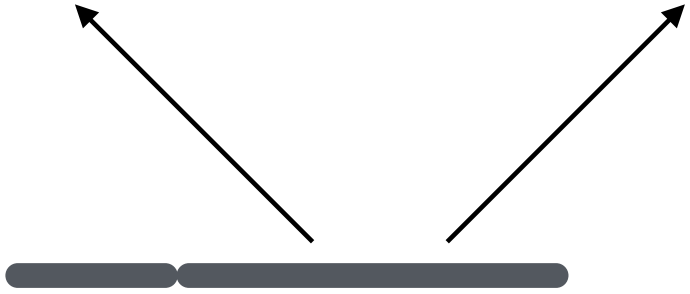


Maternal GM
14 SNPs

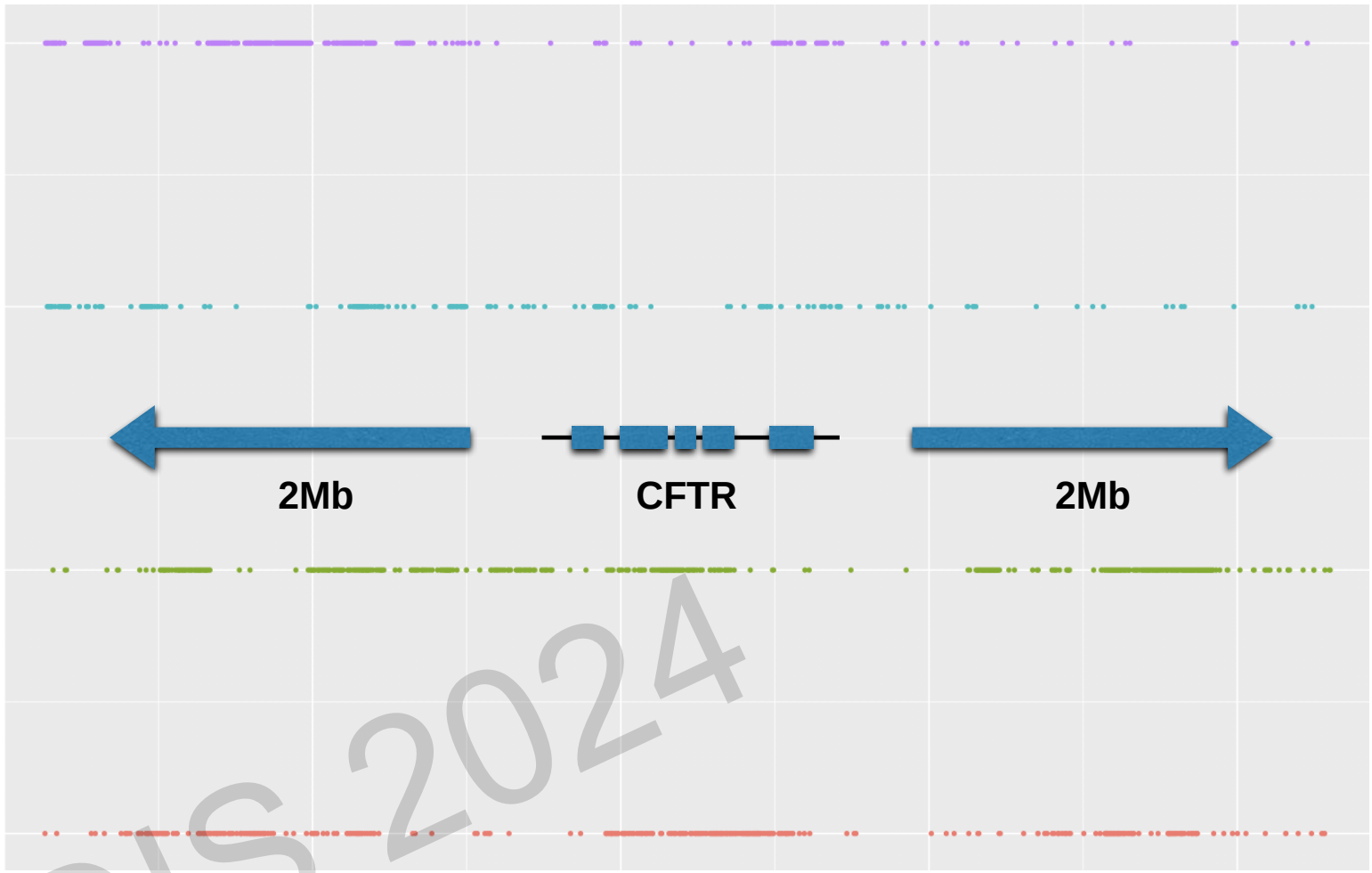
Maternal GF
10 SNPs

Paternal GM
22 SNPs

Paternal GF
22 SNPs



Chromosome 7

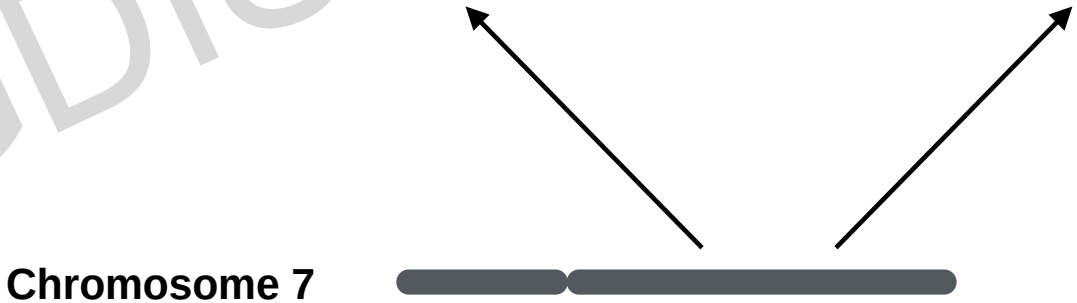


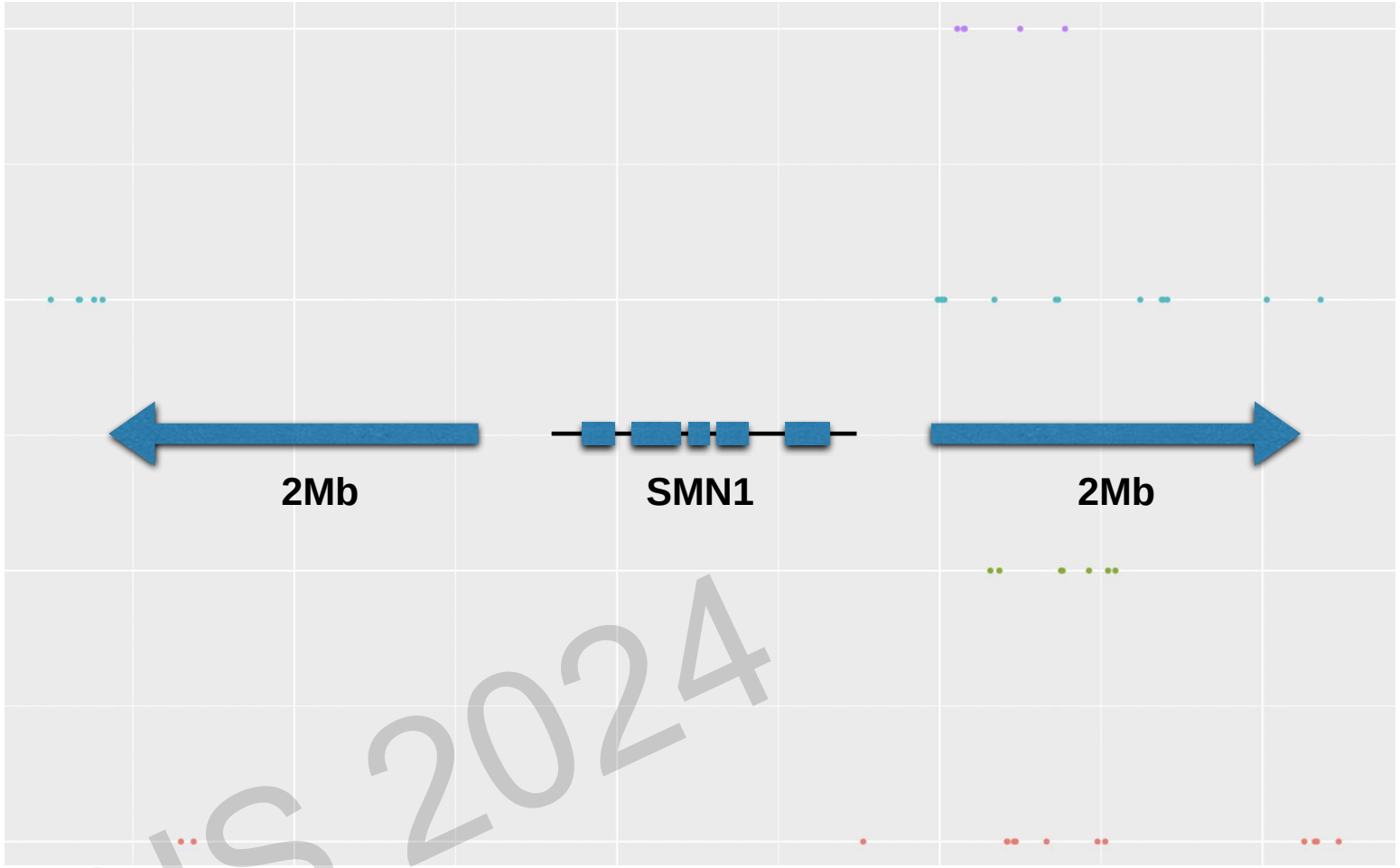
Maternal GM
629 SNPs

Maternal GF
294 SNPs

Paternal GM
785 SNPs

Paternal GF
609 SNPs



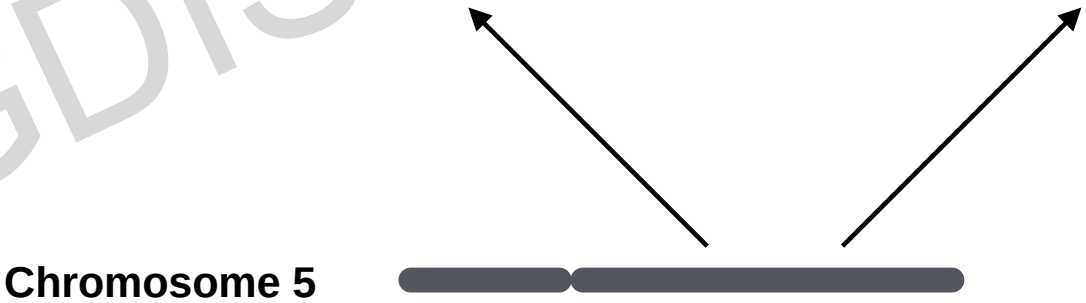


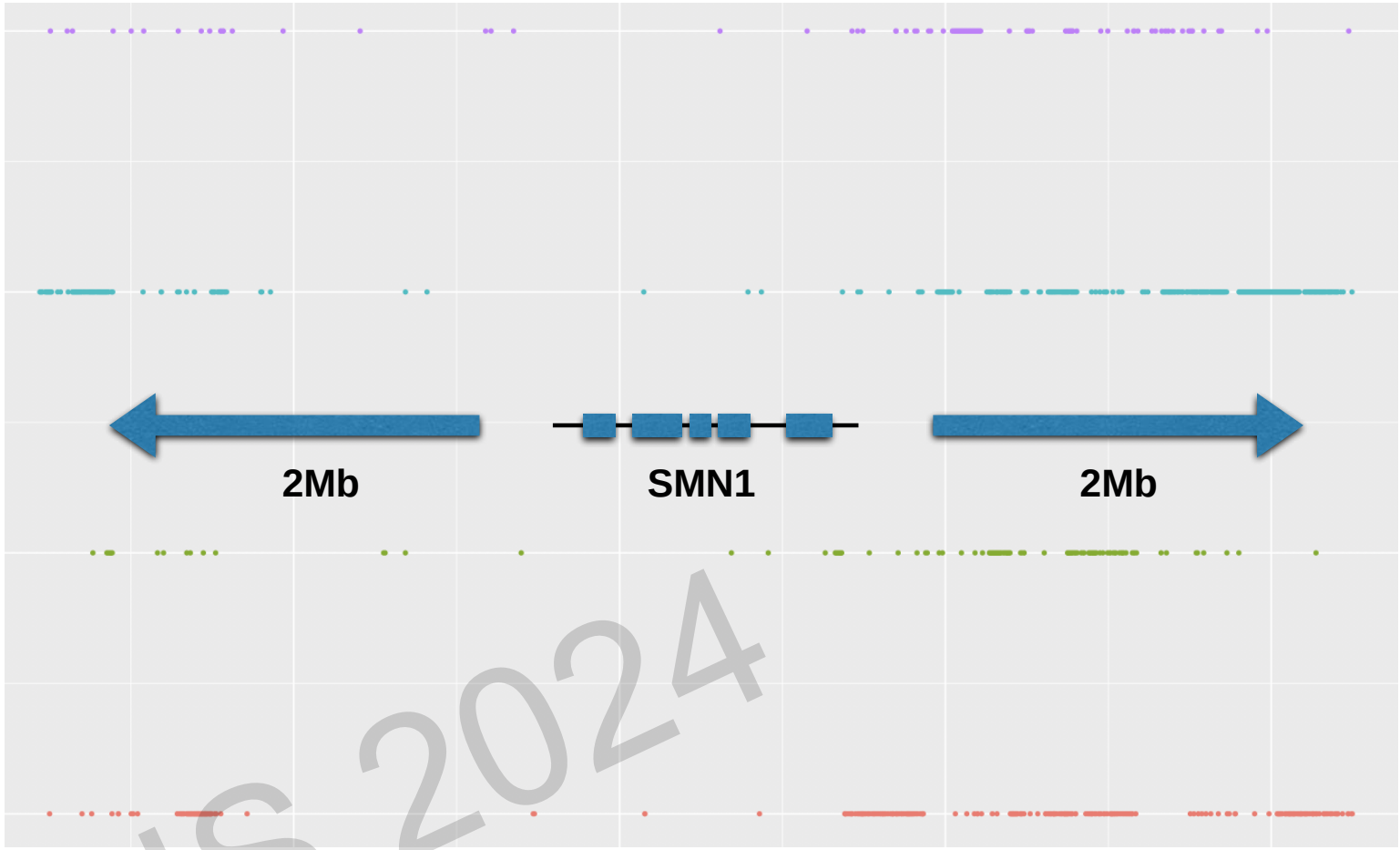
Maternal GM
5 SNPs

Maternal GF
19 SNPs

Paternal GM
7 SNPs

Paternal GF
14 SNPs



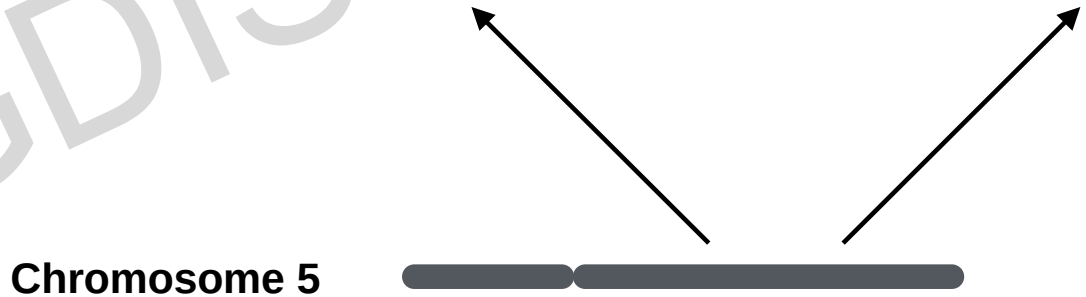


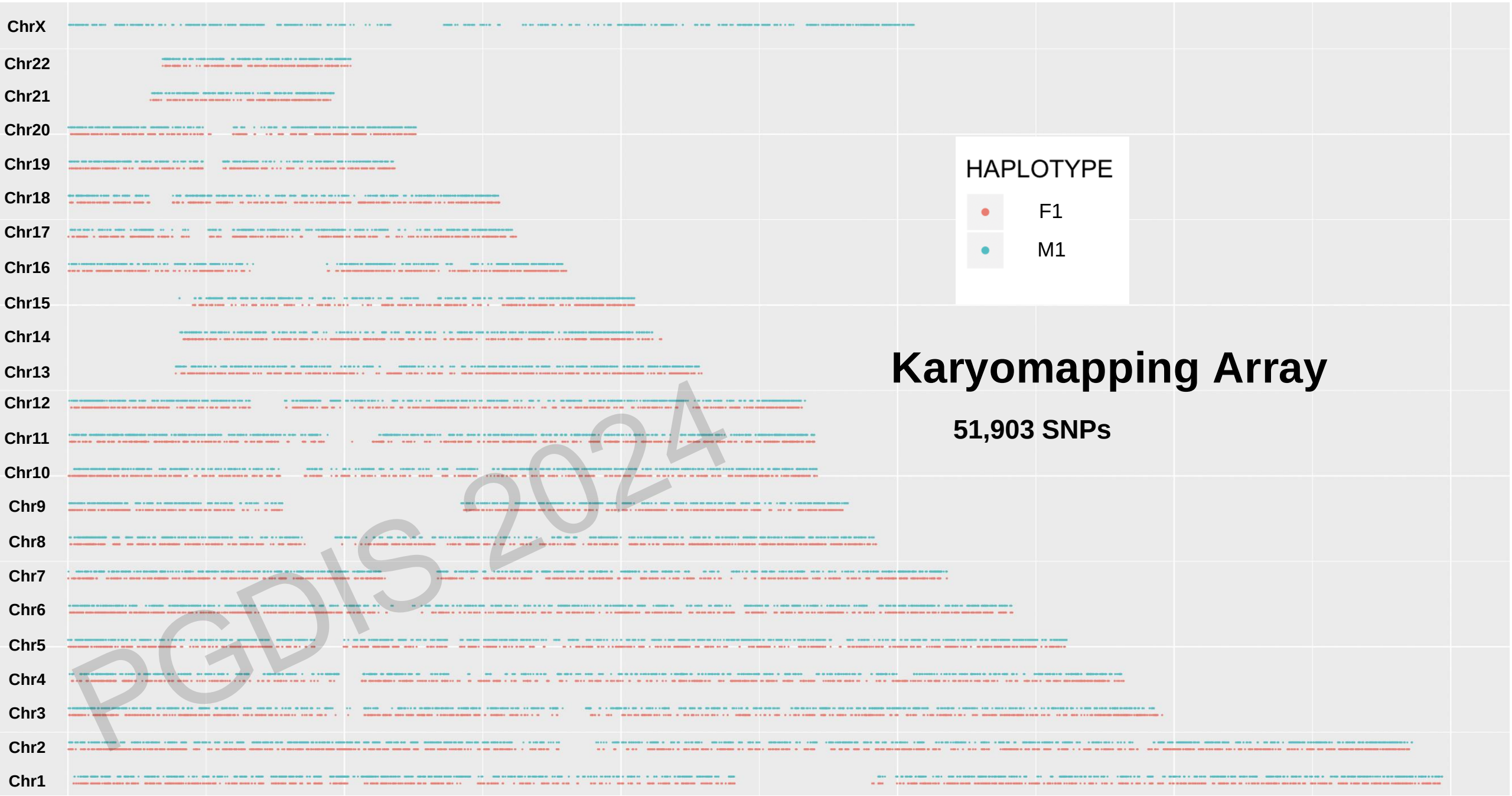
Maternal GM
209 SNPs

Maternal GF
836 SNPs

Paternal GM
246 SNPs

Paternal GF
405 SNPs

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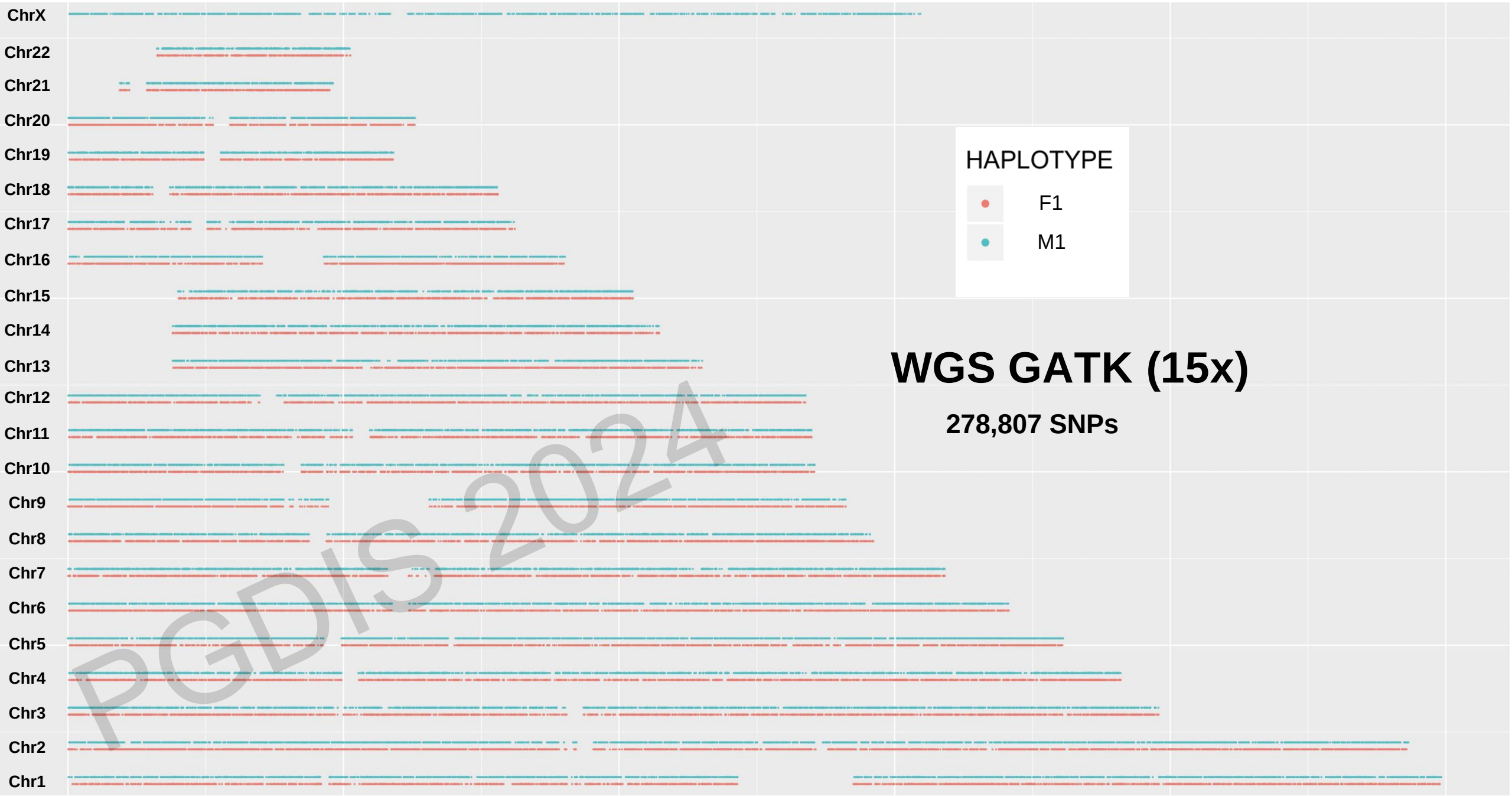
Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
HAPLOTYPE
F1
M1
Karyomapping Array
51,903 SNPs
PGDIS 2024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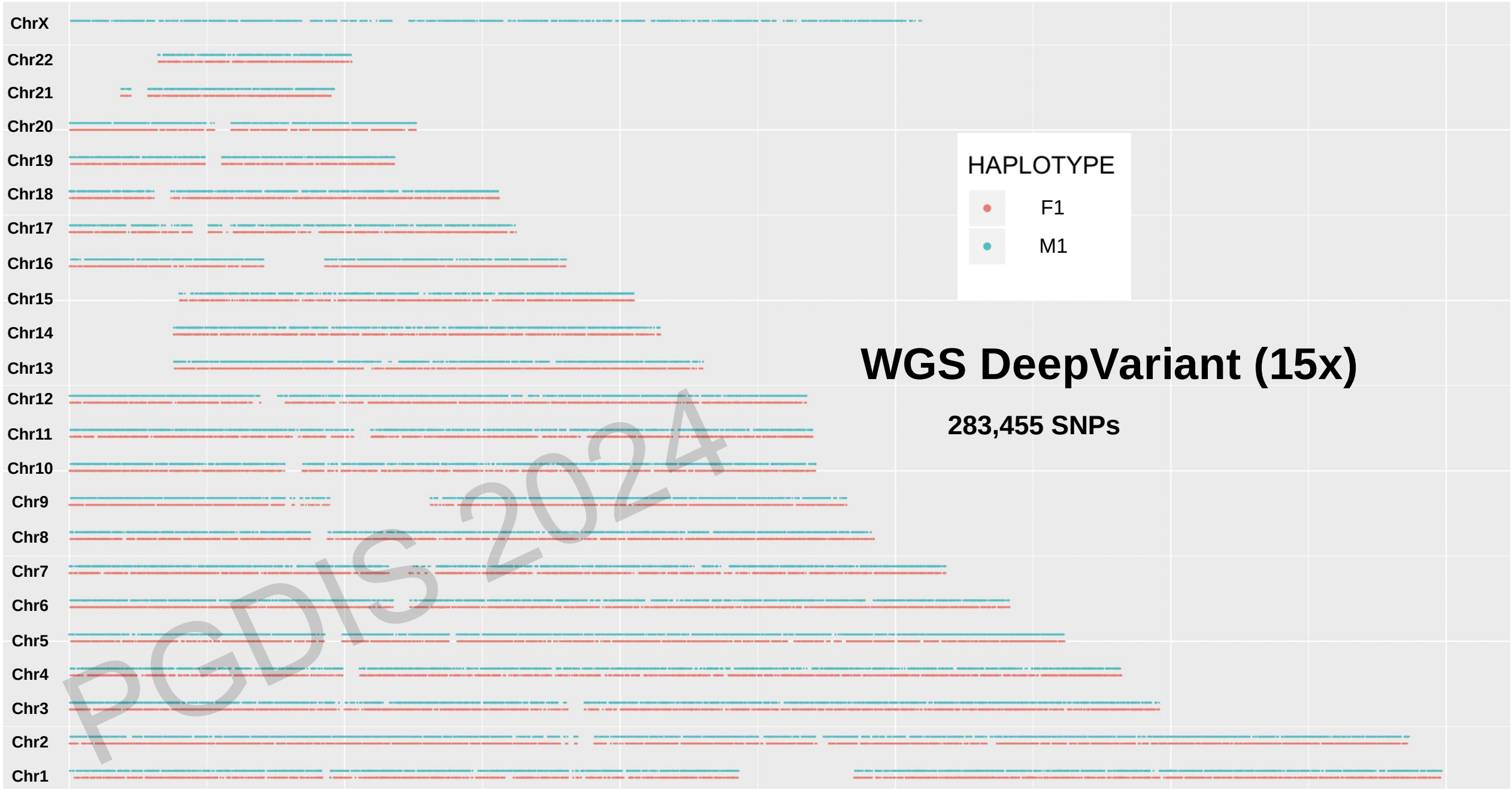
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
HAPLOTYPE
F1
M1
WGS GATK (15x)
278,807 SNPs
PGDIS 2024


ChrX
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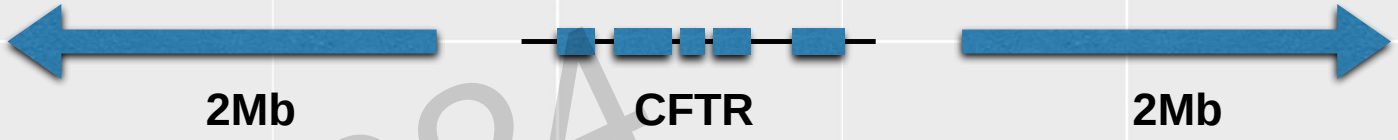
Chr12
Chr11
Chr10
Chr9
Chr8
Chr7
Chr6
Chr5
Chr4
Chr3
Chr2
Chr1
HAPLOTYPE
F1
M1
WGS DeepVariant (15x)
283,455 SNPs
PGDIS 2024

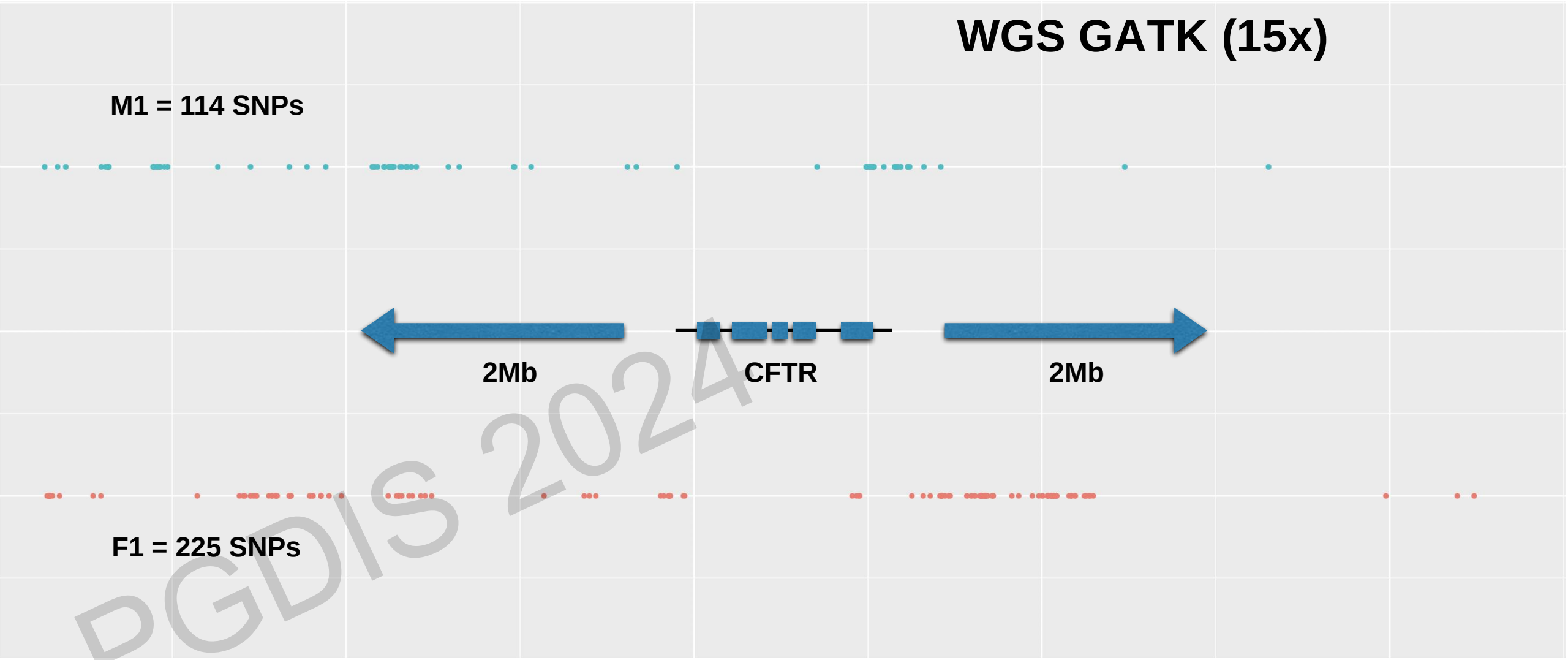
Karyomapping Array

M1 = 12 SNPs

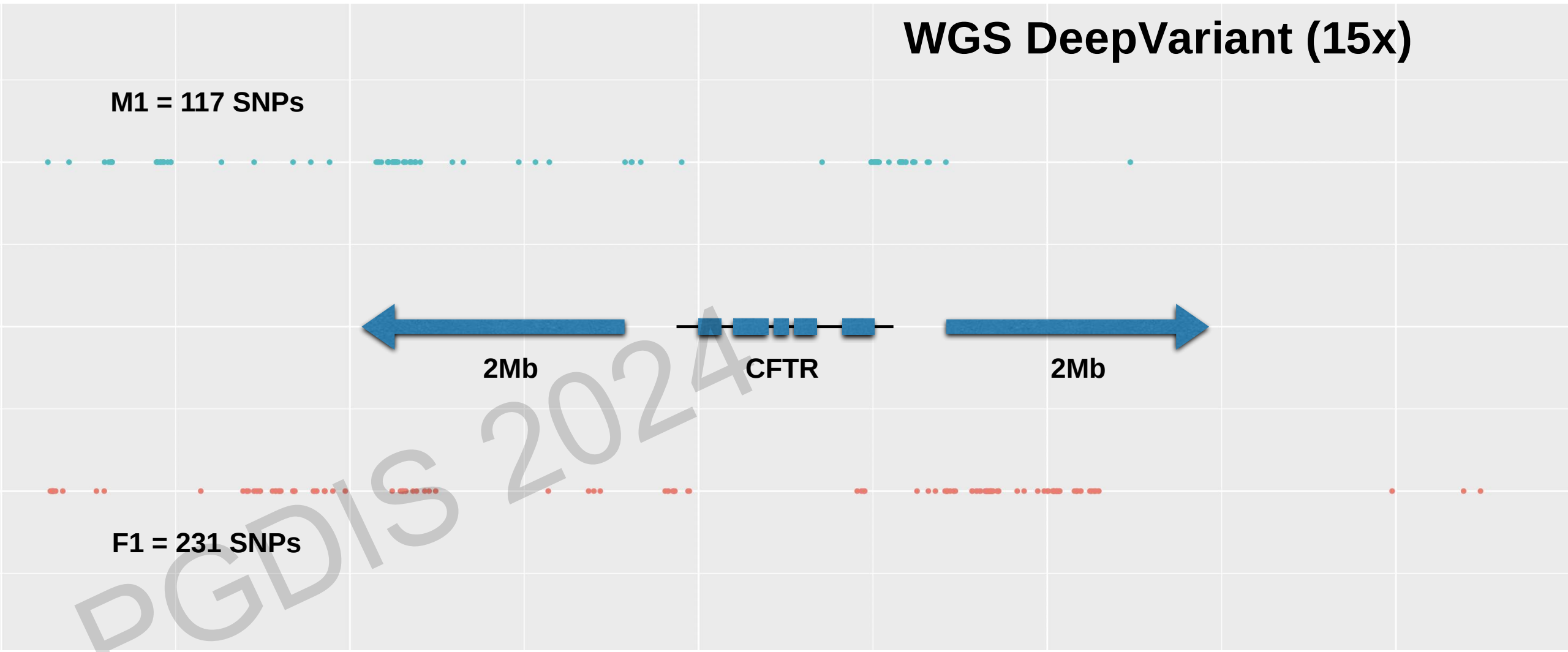


F1 = 21 SNPs

WGS GATK (15x)



WGS DeepVariant (15x)



	Paternal Informative SNPs Median Distance (bp)	Maternal Informative SNPs Median Distance (bp)
WGS	1,179	1,244
SNP Array	27,132	26,432

This is an (expensive) solution in search of a problem!

Possible clinical utility:

- Cases where a meiotic crossover occurs within the gene of interest and cannot be resolved by SNP array

- Greater number of available informative SNPs for specimens with poor amplification or in difficult genomic regions e.g. telomeric/centromeric genes, pseudogenes

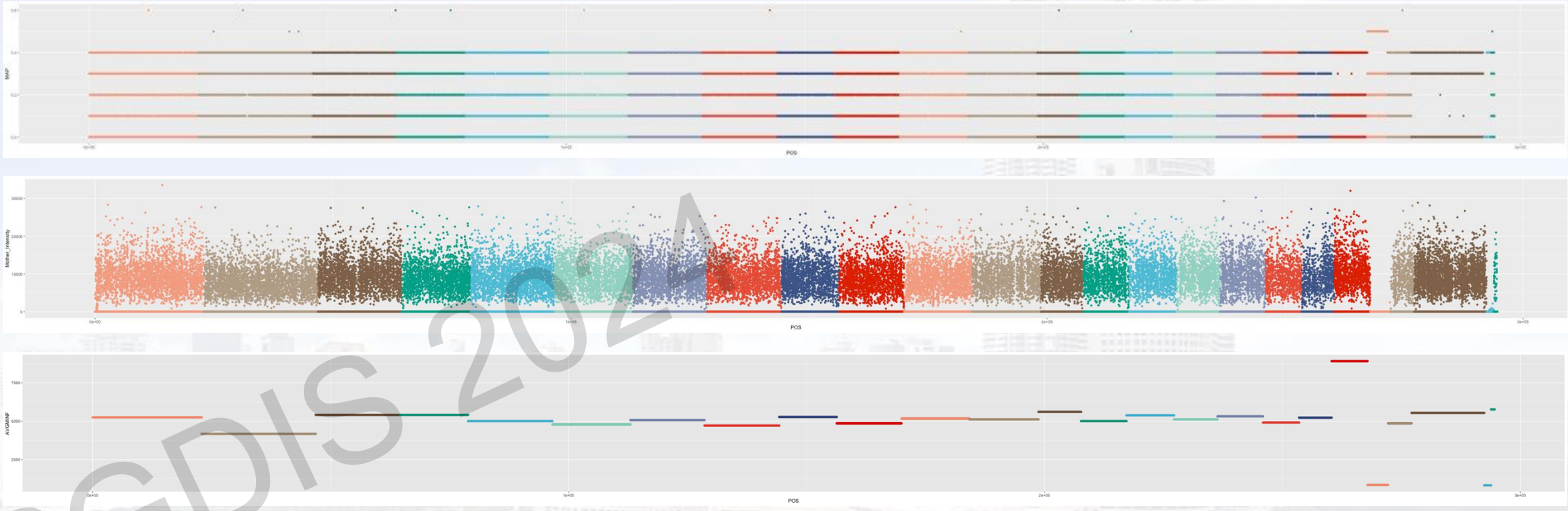
This is an (expensive) solution in search of a problem!

Sequencing provides the same information for PGT-A:

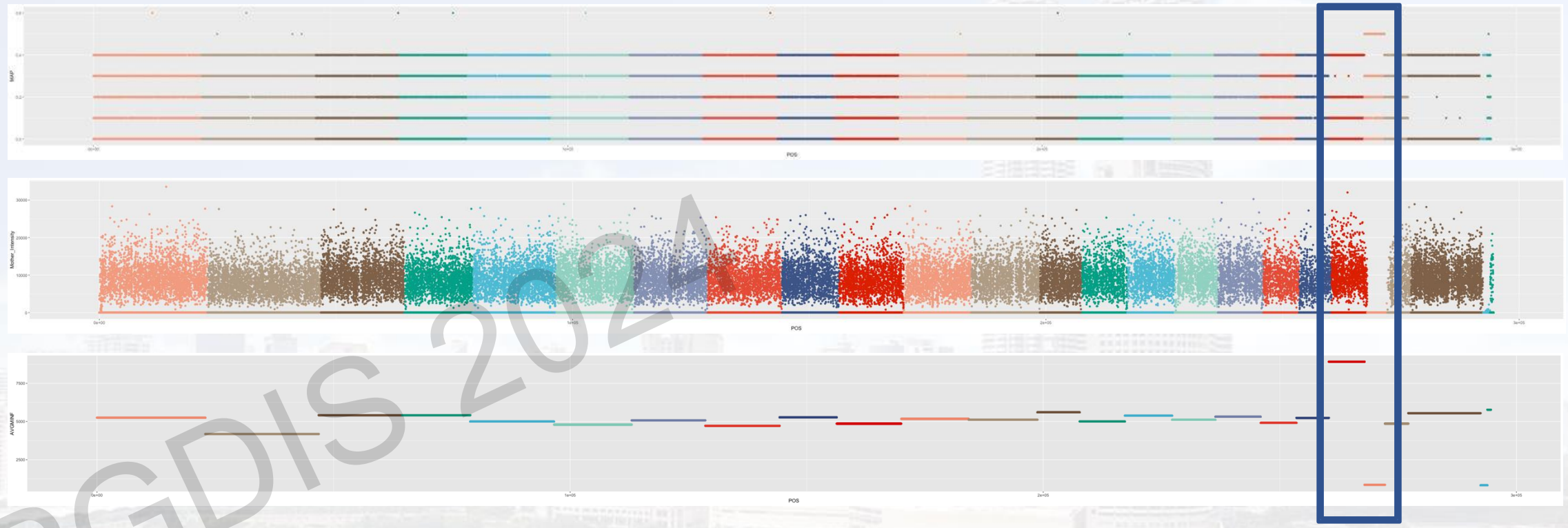
- SNP array combines analysis of SNP haplotypes (including dual haplotype detection for meiotic trisomies) and parent-specific intensity

- WGS provides combined analysis of depth of coverage (intensity) and SNP haplotypes (at sufficient depth)

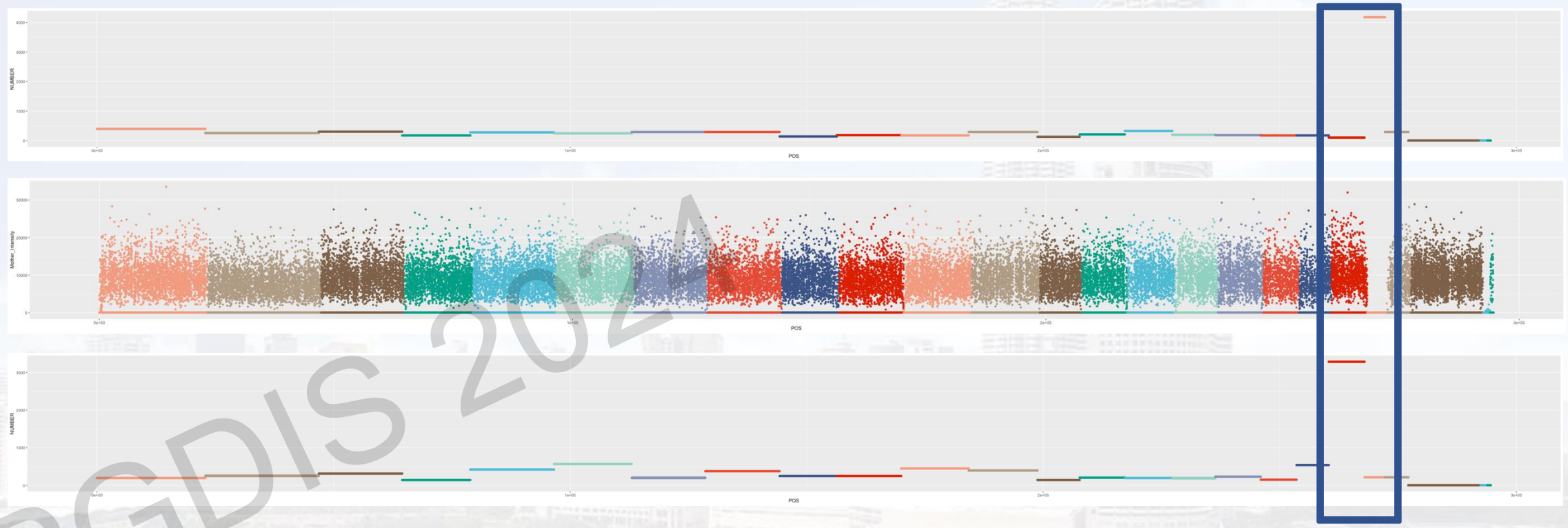
Reference-Free Meiotic Aneuploidy Detection

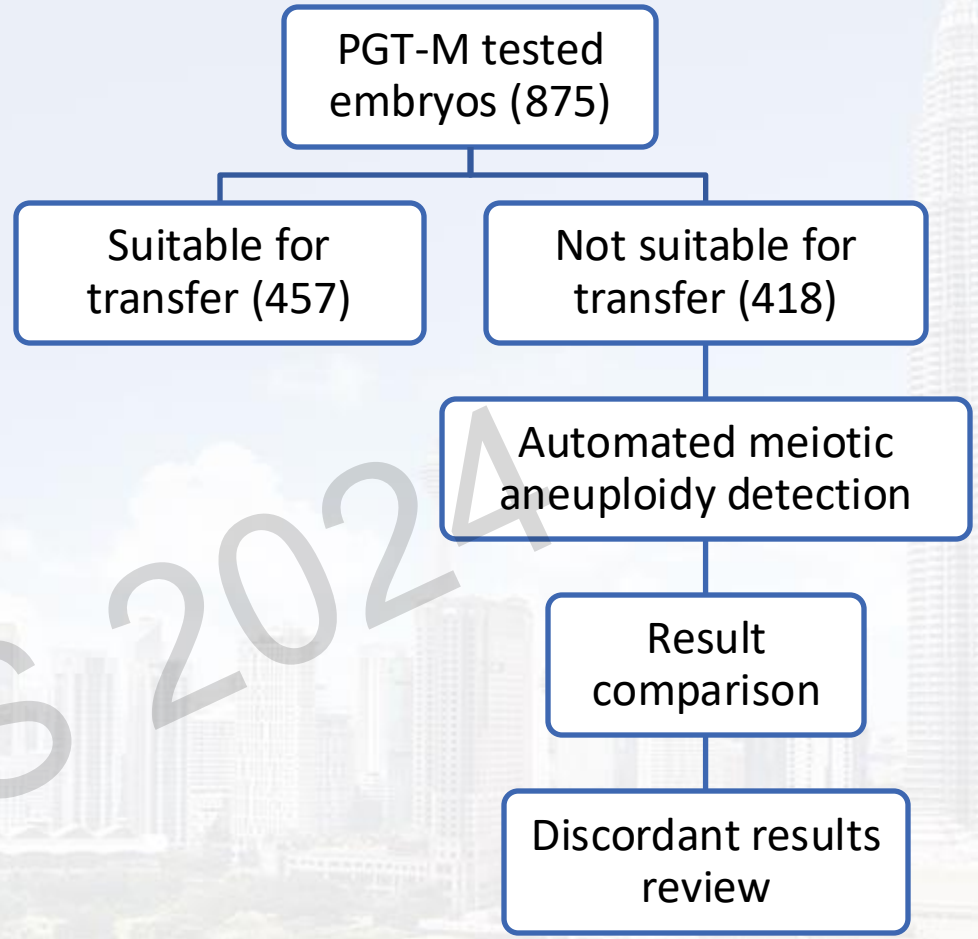


Reference-Free Meiotic Aneuploidy Detection



Reference-Free Meiotic Aneuploidy Detection




PGT-M tested embryos (875)
Suitable for transfer (457)
Not suitable for transfer (418)
Automated meiotic aneuploidy detection
Result comparison
Discordant results review

Reference-Free Meiotic Aneuploidy Detection

High sensitivity for detection of meiotic aneuploidy

Meiotic Aneuploidy

- 131/131 (100%) embryos
  - 158 individual events
  - 3 digynic triploidy
  - 3 genome-wide UPD
- Additional meiotic events detected:
  - 4 trisomies
  - 1 digynic triploid

Reference-Free Meiotic Aneuploidy Detection

High sensitivity for detection of unbalanced PGT-SR embryos

PGT-SR

- 13/13 (100%) unbalanced embryos
- Additional meiotic events detected:
  - 2 trisomies

How could karyomapping benefit from sequencing technology?

- Karyomapping was a solution to the problem of complex, family-specific workups for PGT in the era when references were common

- Autosomal dominant conditions: Affected family members or DNA from deceased individuals typically available

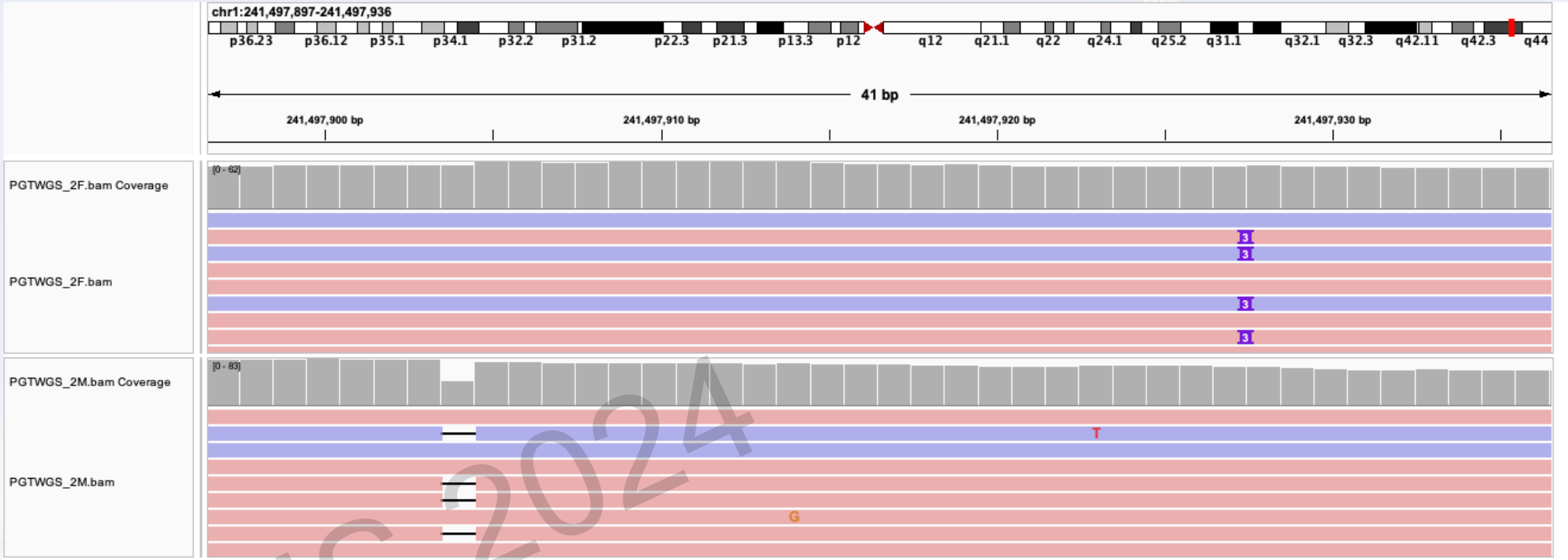
- X-linked and autosomal recessive conditions: Identified with the birth of an affected child

How could karyomapping benefit from sequencing technology?

- Increasingly referrals are received for cases in which a variant is *de novo* or presumed to be inherited but with references unavailable for testing (deceased, overseas, estranged)

- Expanded carrier screening has rapidly increased in utilisation: ~1.9% reproductive couples high risk in a large Australian study

- Whole exome sequencing has the potential to streamline direct variant in combination with confirmation using standard karyomapping analysis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p36.23 p36.12 p35.1 p34.1 p32.2 p31.2 p22.3 p21.3 p13.3 p12 q12 q21.1 q22 q24.1 q25.2 q31.1 q32.1 q32.3 q42.11 q42.3 q44
41 bp
241,497,900 bp
241,497,910 bp
241,497,920 bp
241,497,930 bp
PGTWGS_2F.bam Coverage
[0 - 62]
PGTWGS_2F.bam
PGTWGS_2M.bam Coverage
[0 - 83]
PGTWGS_2M.bam
T
G

How could karyomapping benefit from sequencing technology?

- 22 embryos from 5 families were submitted for Twist Exome 2.0 and Illumina NovaSeq X aiming for ~10Gb output with variant analysis in Dragen v4.0.3.

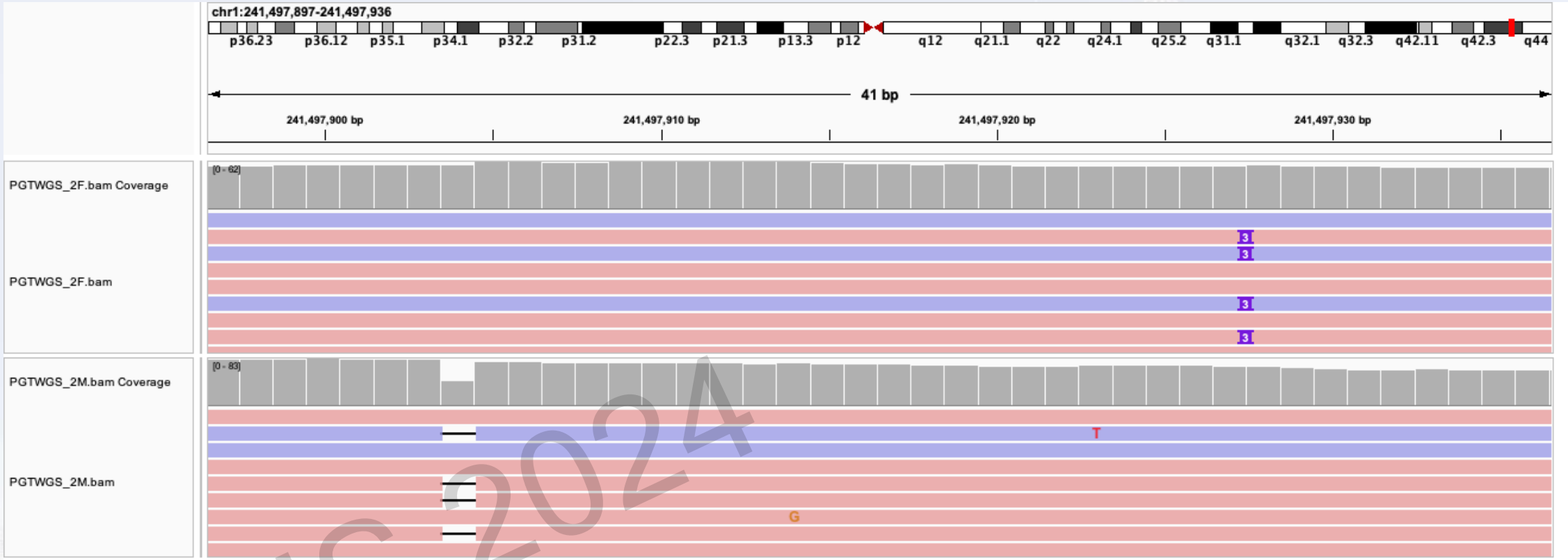
- 22/22 SNVs/indels in 13/13 (100%) of embryos were correctly genotyped across 22 positions using whole exome sequencing, consistent with previous PGT-M results.

- Average coverage >5x was achieved at 96.86% (St. Dev. 1.05%) and >15x at 93.01% (St. Dev. 2.83%) of bases in 22 embryos.

How could karyomapping benefit from sequencing technology?

- Overall allele drop out assessed at expected heterozygous positions (opposite homozygous parental genotypes) was <10% consistent with previous direct variant testing (91.11% average correct heterozygous genotype calls, standard deviation 2.25%).

- Assessment of 10 genes (5 dominant, 4 recessive and 1 X-linked) in a model of de novo variant detection gave results consistent with embryonic haplotypes in 937/946 genotyped positions (99.05% sensitivity of detection).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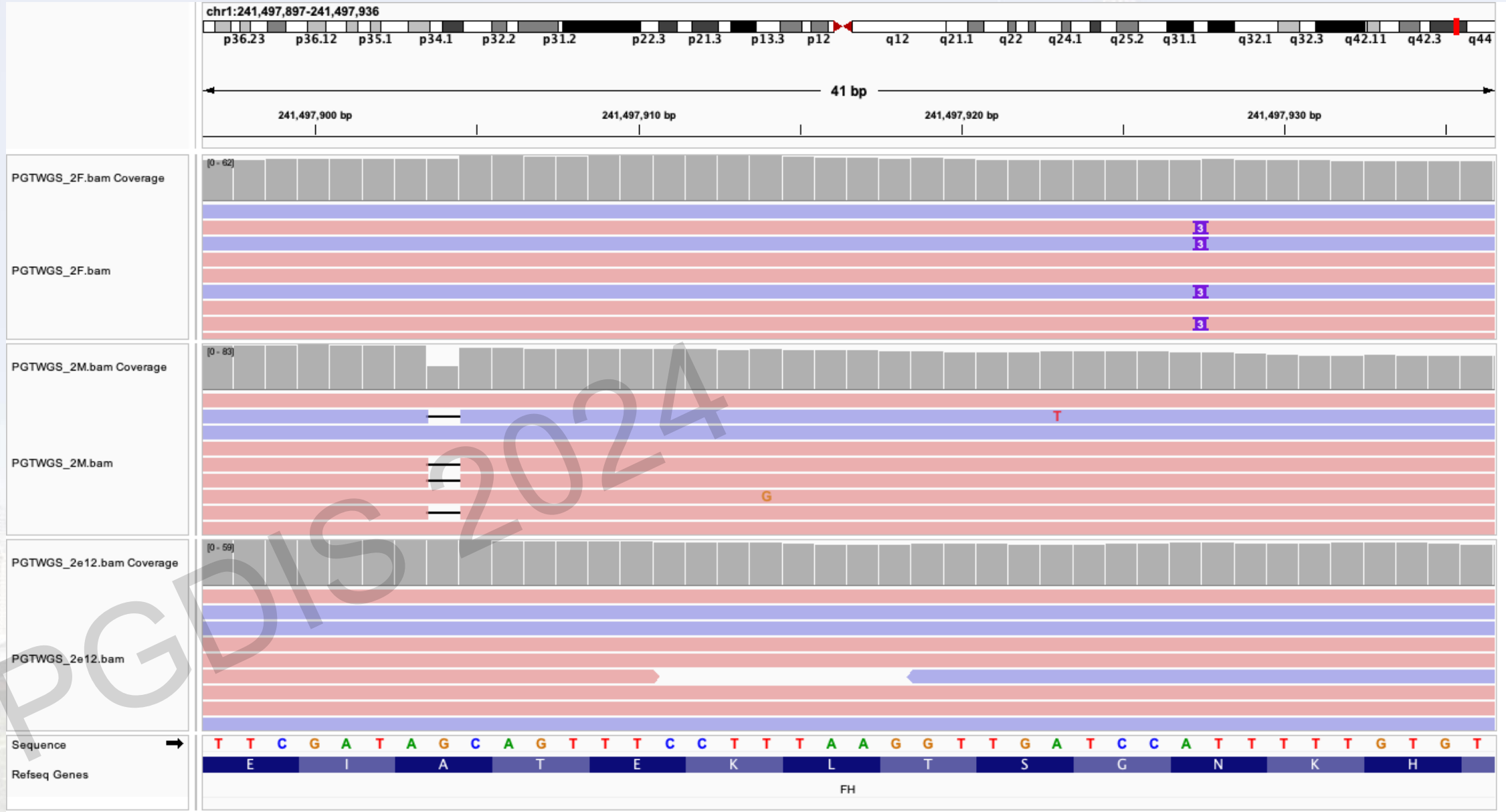
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PGTWGS_2e12.bam Coverage
PGTWGS_2e12.bam
Sequence
Refseq Genes
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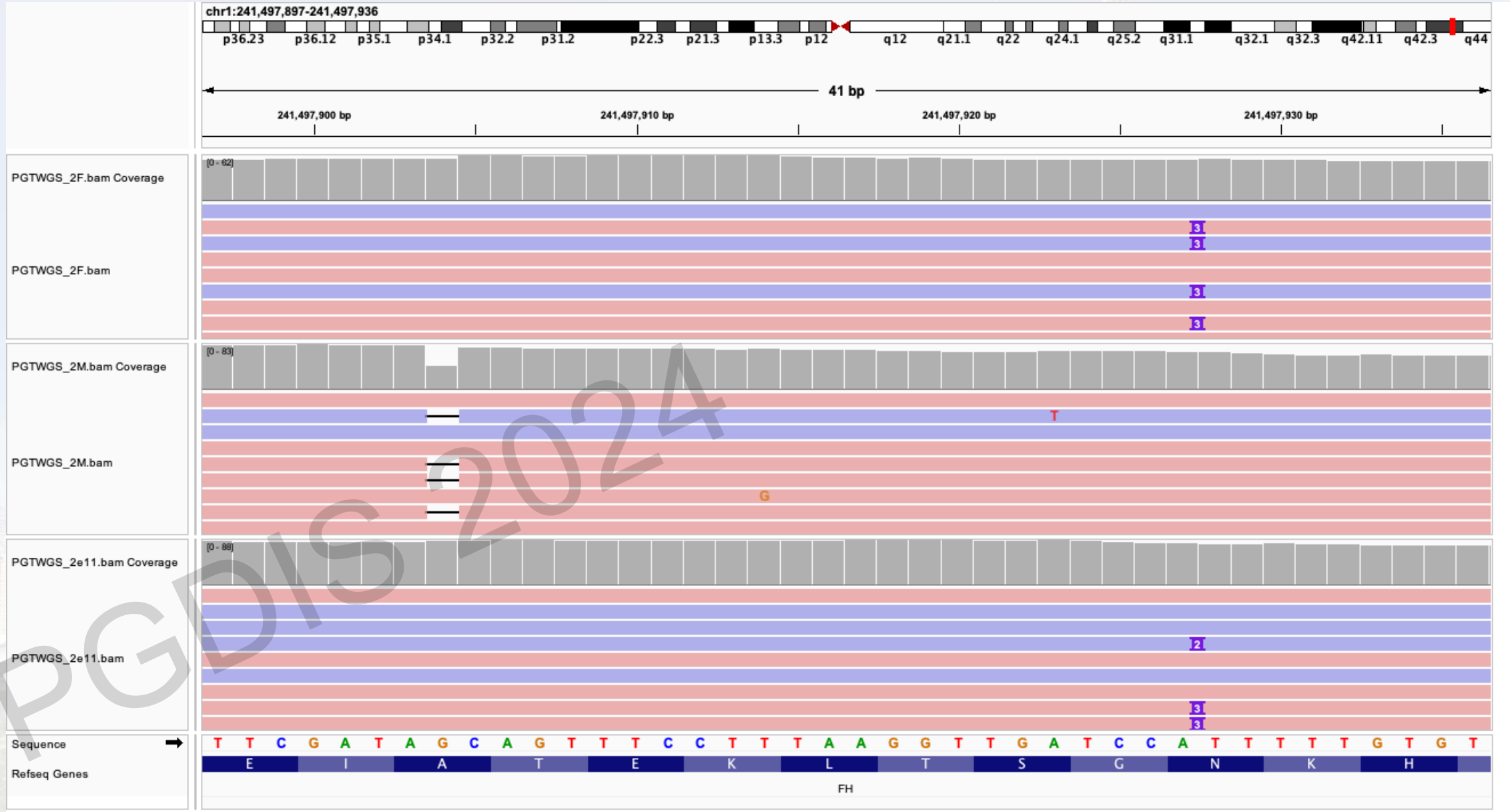
E I A T E K L T S G N K H
FH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PGTWGS_2e11.bam
Sequence
Refseq Genes
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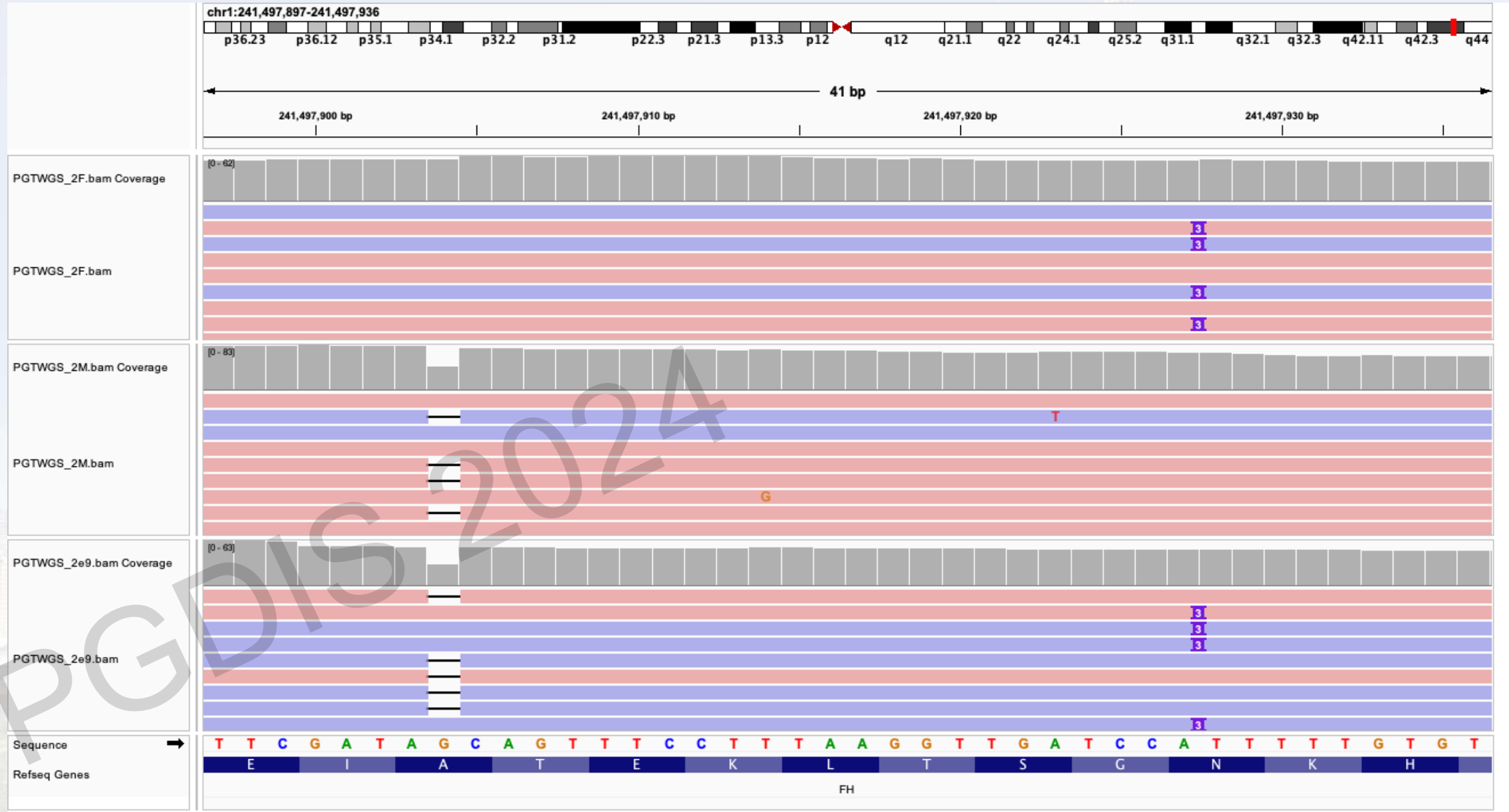
FH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T T C G A T A G C A G T T T C C T T T A A G G T T G A T C C A T T T T T G T G T
E I A T E K L T S G N K H
FH

Conclusions

Karyomapping and its descendants will continue to evolve in the genomic era:

- Providing confirmatory haplotype support in conjunction with direct variant testing following carrier screening or de novo variant detection

- Robust analytical approach for complex genetic pathology requiring long-read sequencing

- Automated aneuploidy detection with high specificity and reduction in false positive calls using parental samples

"Beauty has no obvious use; nor is there any clear cultural necessity for it. Yet civilization could not do without it."

Sigmund Freud, 1930

THANK YOU

Dr. Emily Button, Monash IVF and Repromed Genetics Teams

Prof Alan Handyside

Contact: tristan.hardy@monashivf.com